SPECIFICATION

Please substitute the following paragraph for the paragraph beginning at page 5, line 26:

G. ~~Luizo~~ Liuzzo et al, Circulation, 1999, Nov 23, Vol. 100(21) pp. 2135-9 reported in UA increase in CD4+CD28null cells associated with INF gamma production that was associated with persistent antigenic stimuli. D. E. Newby and K. A. ~~Fox Br Med Bull~~ Fox, Br Med Bull, 2001, Vol. 59, pp. 69-87, reported that use of anti-inflammatory clopidogrel and glycoprotein IIb/IIIa receptor antagonists had positive effect on UA.

Please substitute the following paragraph for the paragraph beginning at page 10, line 10:

One approach that was used to reduce this interference with targeting is the administration of unlabeled targeting antibody prior to the administration of the labeled targeting antibody. This approach was associated with limited and various success (see for examples: M. Helma et al., Cancer Immunol. Immunother. (1998) 47: 39-46, Beatty, B. G. et al., Cancer Res. 49, 1587-1594, 1989; ~~Ahonen~~ A. Ahonen et al., Acta Oncologica (1993), 32, 7/8, pp. 723-7; ~~Schrijvers A. G. H~~. A.H.G.J. Schrijvers et al., ~~J Cancer~~ Cancer Research, ~~(1993)~~ 53, ~~4383-04390~~ 4383-4390, September 15, 1993.

Please substitute the following paragraph for the paragraph beginning at page 10, line 28:

Thus, generally extracorporeal adsorption (ECA) is preferred, in view of the fact that such a method is not associated with administration of additional species to the subject (that may have their own toxicity) and is not associated with increased concentration of TAB, TL

or VL in healthy organs of the subject, associated with clearance, such as the liver and kidney. Generally the removal of one or more of the above species is completed 15 minutes to 48 hours prior to the administration of the TAB-VL or TAB-TL. It should be realized that the Targeting Ligand may be a species that is not an antibody species and its binding to the targeted site in the organism, may be based on non-immunologic binding, as detailed and described in the incorporated references, in particular US ~~patens~~ patents 6,039,946 and 5,753,227 and their counterparts and in Pimm, supra, ~~Gurkavich~~ Garkavij, supra and Goodwin, supra. Thus the targeting ligand may be a ligand that binds non-immunologically to a receptor such as epidermal growth factor receptor, or it may be the peptide hormone somatostatin, that binds to the somatostatin receptor, which is present in high concentration in many tumors. The targeting ligand can also be other targeting proteins or peptides (OTP), or they can be non-peptide targeting molecules such as a drug, for example Atenolol that binds to beta-adrenergic receptor and Haloperidol, that binds the dopamine D2 receptor.

Please substitute the following paragraph for the paragraph beginning at page 12, line 26:

In accordance with the current invention novel devices and methods are provided for the extracorporeal adsorption and removal of molecular and/or cellular tumor immunity suppressor factors (TISF), such as: TGFß, p15E and Sialomucin, suppressor T cells (K.E. Hellstrom and I. Hellstrom, Encyclopedia Of Immunology, I. M. Roitt and P.J. Delves Eds., Academic Press 1992, pp. 1530-1531.), soluble receptor for tumor necrosis factor alpha (s R TNF alpha) and soluble receptor for tumor necrosis factor beta (s R TNF beta), soluble receptors for interleukins 1, 2, and 6 (sR IL-1, sR IL-2, sR IL-6) and soluble receptor for gamma interferon (s R INF-gamma). (M. R. Lentz, Therapeutic Apheresis Vol 3 (1) ~~p~~ pp. 40-

49, 1999 and M. R. Lentz, U.S. Patent 6,231,536 B1. U.S. Patent 6,231,536 provides for extracorporeal adsorption of soluble cytokine receptors by affinity ligands bound to matrix, including the optional addition of treatment with untargeted anticancer drugs. The current invention provides for extracorporeal adsorption of both soluble cytokine receptors as well as other molecular tumor blocking factors and suppressor cells. In accordance with some elements of the current invention the binding of the affinity adsorbent to the matrix in the extracorporeal device is done by binding the adsorbent, when the adsorbent is an antibody to the adsorbed (removed) species, to Protein A that is covalently bound to the matrix of the extracorporeal device, through the Fc part of the antibody, or by binding the affinity ligand, such as Avidin, covalently to the matrix and binding the adsorbent covalently to an affinity counterpart ligand (such as binding Biotin to the antibody adsorbent or to the cytokine adsorbent). The affinity counterpart ligand (Biotin, for example) covalently bound to the adsorbent (adsorbent antibody, for example) is then bound to the covalently matrix-bound Avidin, by non-covalent (by ligand) binding of the Biotin in the biotinylated antibody. The advantage of using Protein A column bound to the adsorbent antibody via the Fc part (domain) of the antibody include: ease of preparation and correct orientation of the adsorbent antibody antigen binding site, for maximal interaction with the antigens (~~Ed~~ E. Harlow and ~~David~~ D. Lane: "Antibodies, A Laboratory Manual, pp: 518-521, Cold Spring Harbor Laboratory Pub, ~~1980~~ 1988).

Please substitute the following paragraph for the paragraph beginning at page 15, line 15:

The MCIFs involved in the etiology and pathogenesis of atherosclerosis (AS) and in particular UA, including those that are increased systematically, are summarized by V.

~~Pasceiri~~ Pasceri and E. T. H. Yeh, Circulation 1999, Vol. 100, pp. 2124-2126. The molecular MCIF species increased in UA include Interleukin 6 (IL-6), IL-2 Receptor, C Reactive Protein (CRP) and TNF alpha. Cellular MCIFs include: CD3+DR+ lymphocytes, CD4+C28-(null) T cells, CD4+ INF gamma+ T cells, Th1 T cells.

Please substitute the following paragraph for the paragraph beginning at page 15, line 22:

Publications reporting the involvement of inflammation processes in AS and in particular AIS include: Z. Reiner et al., Lijec. Vjesn. 2001, Jan-Feb, Vol. 123 (1-2) pp. 26-31. They found that Interleukin 2 (IL-2) was increased in UA and the level of IL 2 was correlated with the level of serum lipids. Increased levels of metalloproteinases and proteolytic enzymes may also be associated with UA. ~~Koukkunen~~ H. Koukkunen et al., ~~Annals.~~ Ann ~~Medicine~~ Med, ~~Vol~~. 2001, Vol. 33 ~~(1)~~ pp. 37-47 reported increased C Reactive Protein (CRP), IL-6, TNF alpha, fibrinogen, Troponin and creatine kinase in UA. ~~K.K. Mizzia~~-Stec et al., Pol Arch ~~med~~ Med Wewn 1999, Aug; Vol. 102(2), pp. 677-84, reported that both TNF alpha pro-inflammatory cytokines and IL-10, anti-inflammatory cytokines, were increased in both stable and unstable angina. A.D. Simon et al., J. Thrombosis and Thrombolysis, 2000, April, Vol. 9. (3) pp. 217-22, found increased IL1-beta and IL-6 in UA. W.H. Lee et al., Exp. Mol. Med. Sept 30, 1999, Vol. 31(3) pp. ~~59-64~~ 159-64, found increased level of CRP in plasma of patients with UA. ~~M.~~ L.M. Biasucci et al., Circulation, 1999, April 27, Vol. 99 (16) pp. 2079-84, found that IL-6 and IL-1Ra were elevated in UA and that their level correlated with the likelihood of a complicated hospital course. G. Caligiuri et al., Circulation, ~~2000~~ September ~~5,~~ 5, 2000, Vol. 102 (10) pp. 1114-9, found that oxidized LDL (OX-LDL) is an antigen that causes the specific proliferation of T cells of patients with UA, thus indicating that OX-LDL

is involved in the etiology and pathogenesis (EP) of UA. A. Mazzone et al., Atherosclerosis, 1999, August, Vol. 145(2) pp. 369-74, found increase of IL- 6 in Ac al., W. H. Lee et al., Exp. Mol. Med., 1999, Sept 30, Vol. 31 930, pp. 159-64 found elevated CRP in MI and UA. ~~M~~ M. Hoffmeister et al., Am J. Cardiol., reported elevated CRP in AIS. D. A. Smith et al., Circulation 2001, August 14, Vol. 104 (7) pp. 746-9, found that IL-10 has a protective role in AS. ~~N.~~ T. Nakajima et. al., Circulation 2001, Feb 5, Vol. 105 (5), pp. 570-75, reported elevated CRP in UA. K.A. Fox et al., Brit Med Bull, 2001, Vol. 59, pp 69-87, pointed out that management of associated risk factors, such as hypercholesterolemia appears to have substantial benefits even during the acute in-hospital phase of Acute Angina. E. Lindmark et al., JAMA 2001, Nov 7, Vol. 286 (17), pp. 2107-13, reported elevated IL-6, CRP and Fibrinogen in UA, particularly a strong independent effect of elevated IL-6 in association with increased mortality in UA.

Please substitute the following paragraph for the paragraph beginning at page 16, line 23:

J. Plutzky, Am. J. Cardiol., 2001, Oct 18, Vol. 88 (8A),pp. 10K -15K, reported elevated CRP, TNF alpha and IL-6 in AIS. ~~Bayes-Genis et. al.~~ L.E. Rabbani, New England J. Med, 2001, Oct 4, Vol. 345 (14), pp. 1057-9, cite Bayes-Genis et al as reporting ~~reported~~ increased level of pregnancy associated plasma Protein A (PAPP-A) in the plasma and in unstable plaques in AIS.

Please substitute the following paragraph for the paragraph beginning at page 16, line 28:

~~H.~~ W.H. Lee et al., Int. J. Cardiol., 2001, Sept-Oct, Vol. 80 (2-3), pp. 135-42, reported increase in CD14 monocytes and HLA-DR+ T lymphocytes in the acute phase of CAD and specifically reported the CD14+ expression on monocytes and percentage of HLA-DR+ T cells are decreased during treatment and that the expression of CD14 represents the activation of monocytes during the acute phase of CAD. W. H. Lee et al., Cardiology, 1999, Vol. 92 (1), pp. ~~11-6~~ 11-16, reported data indicating that the rupture of an atherosclerotic plaque and formation of thrombus may lead to activation of CD40 cells in platelets of patients with AIS.

Please substitute the following paragraph for paragraph beginning at page 17, line 5:

G. Liuzzo et al., Circulation, 1999 Nov. 23, Vol. 100 (21), pp. 2135-9, reported an increase in number of CD4+CD28null T cells in UA. As discussed in Pasceri et al., supra, this increase in T cell population is associated with increased production of INF gamma, that stimulates macrophages to produce metalloproteinases and other proteinase enzymes. P. Aukrust et al., Circulation, 1999, Aug 10, vol 100 (6), pp. 614-20, reported that T cells positive for the CD40L soluble and membrane bound ligand on activated T cells and platelets, particularly in UA, may play a role in the triggering and maintenance of AIS. CD40L cells induced enhanced release of chemoattractant peptide from monocytes, a chemokine involved in the pathogenesis of AS. T. Nakajima et al., Circulation ~~2002~~ 2001, Feb 5, Vol. 105(5), pp. 570-75, reported increased level of CD4+CD28null (null) T cells in AIS. These cytotoxic cells were reported to efficiently kill endothelial cells in vitro and the killing is increased by sensitizing the target cells by CRP. The authors found increased frequency of Perforin and CD16 expressing CD4+ T cells in the peripheral blood, as well as increased CD161 appearance on null cells. Perforin expressing CD4+ cells from UA patients were cytotoxic to endothelial cells In Vitro.

Please substitute the following paragraph for paragraph beginning at page 18, line 3:

G. ~~Luizo~~ Liuzzo et al, Circulation, 1999, Nov 23, Vol. 100(21) pp. 2135-9 reported in UA increase in CD4+CD28null cells associated with INF gamma production that was associated with persistent antigenic stimuli. D. E. Newby and K. A. ~~Fox~~ Fox, Br Med Bull 2001, Vol. 59, pp. 69-87, reported that use of anti-inflammatory clopidogrel and glycoprotein IIb/IIIa receptor antagonists had positive effect on UA.

Please substitute the following paragraph for the paragraph beginning at page 18, line 9:

The improvement that is the subject matter of the current invention is particularly aimed at providing extracorporeal adsorption devices and methods for the effective removal and reduction of MIFs and CIFs (together: MCIFs) species that are etiological in the pathogenesis of AS in particular AIS and most particularly in UA. As mentioned in the background section, the data indicating the implication of MCIFs in etiology and pathogenesis (EP) of AS and AIS are present not only in AS but also in other inflammatory and autoimmune disease (such as rheumatoid arthritis) and may be implicated in non-atherosclerotic vascular diseases such as those associated with autoimmune disease, thus the devices and the methods of the present invention may be utilized in treatment of these diseases as well as in cerebro vascular disease and peripheral vascular disease. Utilization of the methods and devices can also be applicable to inflammatory diseases in general, including inflammation associated with infectious and some neoplastic diseases. MCIFs can be reduced not only by ECA affinity adsorption but also by increased metabolism and clearance, which can be achieved by passive immunization with specific antibodies and including the use of

catalytic antibodies (~~C~~ C. Tellier, Transfus. Clin. Biol., 2002 Jan., Vol. 9, pp. 1-8) including fragments, including synthetic fragments and analogs. Active immunization with immunogenic preparations of MIFs may have utilization in the treatment of more chronic aspects of AS, even though they would not have a role in the treatment of AIS. The MCIFs that are increased in AIS include the following: TNF alpha, metalloproteinases, IL-6, soluble IL2 receptors, CD3+ DR+ T cells, CD4+CD28null T cells, CRP, INF gamma. Utilizing known methods for affinity removal of cells or using ECA affinity adsorption with specific adsorbents of MCIFs such as Mabs, including fragments, or other affinity ligands, such as receptors and synthetic receptors including synthetic receptor fragments and analogs, will lead to removal of MCIFs from the various compartments of the body including biological fluid compartments, such as blood, peritoneal and CSF for example, as well as and in particular from atherosclerotic plaques. The MCIF removal treatment may be combined with drug treatment that is currently used in AIS such as anti-inflammatory and fibrinolytic drugs, for example.

Please substitute the following paragraph for the paragraph beginning at page 19, line 9:

Autoantibodies specific to the β1 Adrenergic receptor have been implicated in the etiology and pathogenesis of Idiopathic Dilated Cardiac Myopathy (IDCM). (~~G.~~ G. Wallukat et ~~al.~~ al, New England J. Med. Vol 347, No 22, Nov 28, ~~2002)~~ 2002, ~~who~~ found that specific removal of these antibodies by the specific synthetic antigen in extra corporeal adsorption (ECA) leads to significant improvement in cardiac performance (utilizing Affina Coraffin® column). Numerous other autoantibodies have also been found in IDCM (~~Muller~~ V. Muller et ~~al.~~ al, Circulation, 2000, Vol 101, pp 385-391). ~~,~~) Of particular interest is the association of IDCM with autoantibodies specific to Oxidized LDL (~~I~~ (I.. Schimke et ~~al.~~ al, J Am College

of Cardiol, 2001, Vol. 38, No 1, pp 178-183) found that following ECA using a sheep anti-human-immunoglobulin antibody, as the adsorbent, the level of autoantibodies specific to oxidized LDL, correlated negatively with lipid peroxide levels (indicator of oxidative stress) as well as with left ventricular ejection fraction. ECA was associated with significant decrease in the level of autoantibodies to the β1 adrenergic receptor. ~~P A~~ P.A. Sobotka et al., Free Radical Biology and Med, Vol. 14, pp 643-647, 1993, reported reduced breath Pentane in Heart Failure, by the use of free radical scavengers (Pentane is produced as a by-product of lipid peroxidation). The use of the above general non-specific adsorbents ("non-specific", with respect to adsorbing specific antibodies), namely: the use, for example, of sheep anti human immunoglobulin antibody, as the adsorbent for the removal (adsorption) of auto antibodies to the β1 adrenergic receptor in the treatment of IDCM, will remove many of the autoantibodies that contribute to the etiology and/or pathogenesis of the disease (autoantibodies to: β1 adrenergic receptor, oxidized LDL, ADP-ATP carrier, alpha and beta cardiac myosin heavy chain isoform, G protein coupled receptors, heart mitochondria).

Please substitute the following paragraph for the paragraph beginning at page 22, line 24:

Protein A-Sepharose® CL-4B obtained from Pharmacia LKB Biotechnology and swollen and washed in accordance with the instruction ~~(Affinity Chromatography. Principles and Methods, Pharmacia Biotechnology Pub., 1991)~~ (Pharmacia LKB Biotechnology, Pub. #18-1022-29, "Affinity Chromatography: Principles and Methods" (1991)), is packed in a column. Preferably the column used is the commercially available Immunosorba®) sold by Fresenius HemoCare Inc. Redmond, WA. The column requires the use of a plasma separator, as is well known and as recommended by the manufacturer: either a "centrifuge" type, such as

Fresenius AS 104 cell separator, Cobe IBM 2997 or membrane type plasma separator, such as Kaneka Sulfox® or Cobe TPE® can be used to separate, on line, the patient's plasma from the cellular elements of blood. While the Immunosorba® column is preferred in some applications, other Protein A Adsorption columns can be used instead. When regeneration is preferred a system including two Immunosorba® columns with a Fresenius Automatic Regeneration unit, Citem 10® are used. When no regeneration of the adsorbent column is needed only a single Immunosorba® column can be used. When it is preferable to use a disposable column, the column utilized for adsorption can be the Fresenius HemoCare Prosorba® column in which the Protein A is covalently bound to a silica matrix. It should be realized that other Protein A columns can be used instead, using various biocompatible matrixes to which the Protein A is covalently bound. (Whenever Protein A is mentioned herein the term includes, peptide fragments of Protein A, including synthetic peptides, that are all known in the art to be used as equivalents of Protein A.) The matrixes used include natural polymers, such as cellulose and dextran, various synthetic polymers and copolymers, such as polyacrylamide, polystyrene and polyvinyl polystyrene copolymer, and silica and silicones. One suitable matrix is heparinized silicone described in D.R. Bennett et al., U.S. Patent 3,453,194.

Please substitute the following paragraph for the paragraph beginning at page 23, line 19:

The configuration of the matrix is also not limited to a particular form, examples of suitable forms are beads (in particular, spherical in shape), fibrous matrixes, macroporous matrixes and membranes, including hollow fibers. One possible device is that of a typical multi-hollow fiber filtration, dialysis or diafiltration design. Such a configuration consists of

a bundle of hollow fibers encased in a tubular housing. The adsorbent is included in the outer space of the hollow fiber, as disclosed in my U.S. Patent 6,039,946, see in particular column 9, lines 9-38 and Figs 2 and 3; the patent uses in the example a chelator as the adsorbent, but in the current invention the adsorbent is Protein A, either in free form, bound to a matrix, particularly by covalent chemical binding. or alternatively the Protein A can be bound to the membrane, to its blood flow side to the side opposite to the blood flow or to both sides, rather then being incorporated in the outer space of the hollow fiber. The Protein A, in either free form, or matrix bound, can also be physically trapped in the membrane, (such as when the membrane is an unisotropic polysulfone membrane, for example the one produced by Amicon). When the Protein A is encapsulated it can be encapsulated in one of the microcapsules or macrocapsules as described in U.S. Patent 5,753,227, in particular column 7, line 53-65, or the double encapsulation described by ~~Markus~~ Marcus et al., American Heart Journal, Vol. 110 No 1, part 1, July 1985, pp. 30-39. In the practice of the invention adsorbents other than Protein A, or in some situations, in addition to Protein A, can be used, that can adsorb CA, CA-NAB, NAB or ATAA. These adsorbents include for example, TAB (that will bind CA, CA2NAB and ATAA) Protein G, C1q bound to antiC 1 q antibody, covalently bound to matrix, Clq covalently bound to matrix (for example, Miro® sold by Fresenius HemoCare) Dextran Sulfate (for example, Selsorb® sold by Kaneka Corp.) and the adsorbent disclosed in Kuroda et al., U.S. Patent 4,627,915. Optionally, the Protein A extracorporeal column adsorbent can incorporate specific adsorbents, as detailed in U.S. Patent 5,753,227, column 10, line 51 to column 11, line 9. In the application of this method in accordance with the current invention an intact antibody, or an antibody fragment containing Fc fragment, and specific to a molecular species, desired to be removed, in accordance with the current invention, is bound to the protein A in the column by ~~none~~

~~covalent~~ non-covalent binding, following procedures known to those skilled in the art (see for example, E. Harlow and ~~D Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Pub., pp. 511-527, 1988, and Affinity Chromatography, Principles and Methods, Pharmacia LKB Biotechnology Pub. # 18-1022-29,~~ D. Lane, "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory Pub., pp. 511-527, 1988, and Pharmacia LKB Biotechnology, Pub. #18-1022-29, "Affinity Chromatography: Principles and Methods" (1991) pp. 47-52). In accordance with the present invention the antibodies bound to the protein A in the ECA column are one or more of the following specific antibodies (SA): Antibody to TAB, antibody to CA (for example TAB) antibody to NAB, (anti idiotypic antibody) (antibody to ATAA (e. g. anti idiotypic antibody), CA-NAB, NAB, antibody to tumor blocking and tumor immunity suppressor factors and suppressor cells such as: TGF β, pl5E, Sialomucin, sR TNF alpha, sR TNF beta, sR IL-1, sR IL-2, sR IL-6, sR INF gamma, TH2 T cell epitope. The specific antibodies coupled to Protein A are crossed linked to the Protein A by a bifunctional coupling reagent, preferably dimethylpimelimidate (DMP), or by another coupling reagent, in accordance to Harlow and Lane Supra. In the Example, the organism being treated is a human, the adsorption system used is the Fresenius system containing two Immunosorba® columns and a Citem 10® regeneration unit. Each column contains 62.5 ml of Protein A, which is covalently bound to a cross-linked beaded Sepharose matrix. Plasma is first separated from blood cellular elements, using Fresenius AS 104 Cell Separator. To the Protein A in the column, are bound, through the Fc binding site of the Protein A, intact antibodies to at least one of the above specified chemical or cellular species (TAB, CA, NAB (anti idiotypic antibody), ATAA (anti idiotypic or anti isotypic antibody), CA-NAB, TGF β, P15E, sialomucin, sR TNF alpha, sR TNF beta, sR IL h1, sR Il 2, sR IL6, sR INF gamma, TH2 T cell epitope. In the EXAMPLE 1, the matrix bound Protein A is

bound to the Fc part of antibody ZCE-025 specific to CEA antigen (to adsorb circulating CEA and to adsorb ATAA that is specific to antibody ZCE-025. Mab CH-255 is bound via Fc to Protein A in the ECA column to adsorb the VL L-SCN-C6H4-CH2-EDTA-In.

Please substitute the following paragraph for the paragraph beginning at page 33, line 17:

The subject being treated for therapeutic or diagnostic purposes is a human subject with a malignant melanoma tumor which is positive for the p97 surface glycoprotein antigen. The TAB is the monoclonal antibody 96.5 specific to p97. The TAB is labeled with 131 I and conjugated to Biotin in accordance with U.S. Patent 6,251,394. See column 8, line 66 to column 9, line 19:

"The monoclonal antibody was the 96.5 (mouse IgG2a) specific for p97, a cell surface glycoprotein with the molecular weight of 97,000 present on 60-80% of human melanoma. The tumor model has been described in detail (~~Ingvar. C. et al., Nucl. Med 30. 1989, 1224~~ C. Ingvar et al, Nucl. Med., Vol. 30, 1989, pp. 1224-34).

2. Conjugation and Labeling of Monoclonal Antibodies.

The monoclonal antibody 96.5 (330 ug) was labeled with s 37 MBq iodine-125 (^{125}I), using the Chloramine-T method. By elusion on a Sephadex G25 column (Pharmacia PD 10) the fraction containing the labelled protein was collected and used for the conjugation. The labelling efficiency of the ^{125}I 96.5 was around 70%. The radiolabelled monoclonal antibody was conjugated with biotin by mixing 500 ug of antibody with 41 ug of N-Hydroxysuccinimido-biotin (NHS-biotin) in 0.1 M NaHCO3, 0.15M NaCl with 10% DMSO. The mixture was

incubated for 1 h at room temperature, followed by overnight incubation at 4° C. The ^{125}-McAb-biotin conjugate was separated from free biotin-reagent by gelfiltration on a Sephadex G25 column, equilibrated with PBS (10 mM Sodium phosphate, 0.15 M NaCl, pH 7.3). The conjugate was stored at 4° C until used."

Please substitute the following paragraph for the paragraph beginning at page 36, line 13:

The use of Avidin-Biotin combination, wherein the FIRST adsorbent is Avidin, was proposed by ~~J Tennval~~ J. Tennvall et al., Cancer Suppl., Vol. 80, number 12, pp. 2411-2418, December 15, 1997, for the removal of ATAA, by adsorbing to the Avidin in the ECA column biotinylated TAB. In accordance with the current invention, this approach can be utilized to remove any molecular or cellular TSF, by binding to the Avidin in the ECA column one or more biotinylated antibodies specific to one or more molecular or cellular TSF. It should be realized that the method of utilizing a SECOND specific adsorbent, bound non-covalently (e.g. bound by ligand) to a FIRST adsorbent that is covalently bound to a matrix, is not limited to Protein A-specific antibody or to Avidin-Biotinylated specific antibody pairs. Various pairs of affinity ligands can be used as the FIRST adsorbent and the SECOND adsorbent. The FIRST adsorbent, for example, could be an antigen or a hapten covalently bound to a matrix, or dinitrophenyl (DNP) bound to a matrix, preferably through a spacer, or a carrier molecule, such as albumin (e.g., albumin-DNP conjugate bound to the matrix). The SECOND adsorbent can then be a hybrid antibody specific to both DNP and the TSF. The FIRST adsorbent can be an antibody to DNP and the SECOND adsorbent an antibody to TSF covalently bound to DNP. The FIRST adsorbent can be an enzyme bound covalently to the matrix in the ECA and the SECOND specific adsorbent will then be an enzyme substrate or

enzyme inhibitor conjugated to an antibody specific to TSF (or to another molecular or cellular species targeted for removal). Similarly, any of other known affinity pairs such as, for example, those listed in U.S. Patent 6,251,394, column 7, lines 54 to 67, can be used, including Lectins/saccharide residues (e.g. lectin from Sambueus nigra/beta-D-gal(1-4)-D-glc), Enzyme/enzyme inhibitors (e.g. D-Alanin carboxypeptidase from B.subtilis or E.coli/ 6-amninopenicillanic acid or p-aminobenzylpenicillin, or e.g. Dehydrofolate reductase/aminopterin or amethopterin), or protein/co-factors. (e.g. Intrinsic factor/vitamin B12 or cobalamin). It will be understood that with any affinity binding pair, either one of the affinity binding pair may be the FIRST adsorbent and the other of the pair may be the SECOND adsorbent.

Please substitute the following paragraph for the paragraph beginning at page 37, line 18:

These EXAMPLES are identical to EXAMPLES 4-8 except that the TL is the anti-cancer drug calicheamicin. The subject treated is a human being having an acute myeloid leukemia positive for the CD33 antigen. The TAB is recombinant engineered human anti-CD33. The TAB is conjugated to the calicheamicin in accordance to ~~L~~ L. M. Hinman et al., Cancer Research, Vol 53, ~~pp. 336-3342,~~ July 15, 1993, pp. 3336-3342. The dose of administered TAB-Calicheamicin conjugate is 6-9 mg protein/ m2 ~~(E L Sievers et al., Blood Vol. 93 (11), June 1, 1999).~~ (E.L. Sievers, et al., Blood, Vol. 93 (11) June 1, 1999, pp . 3678-84.)

Please substitute the following paragraph for the paragraph beginning at page 38, line 20:

A conjugate of Avidin with Mab ZCE-025, which is specific to CEA antigen is prepared according to H. P. Kalofonos et. al., The Journal of Nuclear Medicine, Vol. 31, No 11, pp. 1791-1796, 1990., except that the Mab ZCE-025 is substituted for Mab HMFG1. The subject being treated is having a tumor positive to the CEA antigen and has circulating CEA antigen in his blood. As determined by standard methods known in the art and available commercially. In Step 1: The subject is administered intravenously (1 -4 min) 0.1 mg protein/Kg to 2 mg protein/Kg of the Mab-Avidin conjugate. In Step 2: The subject is treated with ECA with a Biotin Adsorbent Column (BAC). The BAC ECA starts 0.5h to 48 hours, preferably 2-24 hours after the administration of the Mab-Avidin conjugate. 1-4 volumes of plasma are treated. The length of the ECA is 1-4 hours. The flow rate is 20 ml/min to 50 ml/min. For its use as the adsorbent in the ECA Biotin is covalently bound to a macromolecular carrier, such as human serum albumin (HSA), using the method described in U.S. Patent 6,251,394, except that the albumin is substituted for the antibody. Albumin labeling with Biotin can also be done, following the method described in Harlow and Lane supra, pp. 340-341, substituting the HSA for antibody. The Albumin-Biotin is covalently bound to cyanogen bromide Sepharose 4B beads available from Pharmacia. As an alternative to binding of Albumin-Biotin to cyanogen bromide activated Sepharose, the binding of the biotinylated albumin to Sepharose can be done by using Avidin-Biotin binding. The albumin is biotinylated, so as to have sufficient Biotin moieties conjugated to the albumin, thus leaving sufficient number of Biotin molecules in the ECA column to be available to bind to Avidin in the Mab-Avidin conjugate. In Step 3, the subject is given intravenously 0.5-10 mg protein of the conjugate Biotin-Human Serum Albumin (HSA)-^{131}I in 1-5 ml volume of 8.4% Sodium bicarbonate. Human serum albumin is directly Iodinated with ^{131}I according to E. Harlow and D. Lane: ~~Antibodies~~ Antibodies, A Laboratory Manual, Cold Spring ~~harbor~~ Harbor

Laboratory Pub., pp.324-329, except that Human Serum Albumin is substituted for the antibody for the direct Iodination. The preferred Iodination is by the Chloramine T method (~~E~~ E. Harlow and ~~D~~ D. Lane supra, 328-329).

Please substitute the following paragraph for the paragraph beginning at page 41, line 18:

The affinity adsorbent for the TSF (including TISF, including molecular and cellular TISF) as well as the affinity adsorbent for MCS, such as, for example, an antibody to Botulinum Exotoxin or an antibody to Tetanus Exotoxin (including antibody fragment, including synthetic fragments and synthetic or genetically engineered antibody binding site analogs.) Other affinity adsorbents can also be used, such as, for example, binding sites of Botulinum Toxin (N. M. Bakry et al: Infec. Immunol., Vol 65 (6) pp 225-32, 1997), including synthetic binding sites and synthetic analogs of binding sites. The affinity adsorbents can be incorporated in the ECA device, using any of the methods disclosed in U.S. Patents 6,039,946 and 4,813,924, such as being covalently bound to a matrix (such as a natural polymer or synthetic polymer, as well as silica. Alternatively, the affinity adsorbent can be encapsulated in a microcapsule or macrocapsule, or can be incorporated or attached to a macrosphere or microsphere. When the affinity adsorbent is an antibody or an Fc containing antibody fragment, it can be bound to Protein A or Protein G through Fc non-covalent (by ligand) binding. When the adsorbent is a biotinylated, or Avidin-bound affinity adsorbent, it can be bound non-covalently (by ligand) to Avidin or Biotin, respectively, which are covalently bound to a matrix in the column. Other utilizations include affinity labeling for ECA of specific cellular and/or molecular species that are collected or harvested for therapeutic use, in the same subject, from which they are removed, or in another subject: For example, removal

of cytotoxic T cells and natural killer cells (lymphokine activated killer cells (LAK) and tumor ~~infiltrating _lymphocytes~~ infiltrating lymphocytes (TIL)), for in vitro activation and expansion, followed by re-administration to the subject, in the treatment of cancer, e.g. in vitro stimulation of harvested T and NK cells by treatment with lymphokines, in LAK or TIL cell treatment of cancer (See for example J. J. Sussman et al., Ann Surg Oncol, Vol 1 (4), pp. 296-306, 1994 and ~~S-A~~ S.A. Rosenberg et al., Science, Vol. 233(4770), pp. 1318-21, Sept 1986). The only requirement for such affinity adsorption harvesting is the availability or the ability to produce specific affinity labeling reagent that is specific to the target molecular or cellular species, and the ability to conjugate the targeting species to an affinity marker, such as Avidin (or Biotin), for example. Markers of LAK and TIL cells include, for example, CD3+, CD56+, DM1, VGO1 and LAK1. (~~R-E~~ R.B. Herberman; Encyclopedia of Immunology, I M Roitt and P J Delves, Eds. Academic Press 1992, pp 1013-15.) It would be obvious that the method can be modified by, for example, the use of Biotin for affinity labeling and use of Avidin as adsorbent in the ECA, or the use of affinity pairs other than Avidin Biotin, for example: anti-hapten antibody – hapten, enzyme-substrate and the like. For example, in LAK and/or TIL based treatment, an antibody, such as biotinylated antibody specific to LAK and T cell markers, are used as the adsorbents or a tumor specific antigen (or a synthetic analog of the tumor specific antigen) to which the TIL has affinity, can be used as the adsorbent. One significant advantage of the proposed affinity labeling, is that it can be possible to use one single ECA device (Avidin-ECA, Biotin-ECA, for example) to remove many different species from the subject, by using different specific affinity labels targeted to the species to be removed and adsorbing them on the single device used for the ECA step of the method. Alternatively, the different species can be removed at different times or at the same time. The affinity label specific to the species to be removed can either be administered

to the subject being treated prior to the ECA step, or preferably the affinity labels are not administered to the subject being treated but are incorporated in the ECA device itself. For example, when the affinity labels are biotinylated antibodies specific to the species to be removed, and the device is an Avidin-ECA device.

Please substitute the following paragraph for the paragraph beginning at page 43, line 9:

For example, if the species is TAA it can be targeted by TAB-Avidin or by Anti-idiotypic antibody to TAA and removed by a Biotin-ECA. If, the species is oxidized LDL it can be affinity labeled by administering a conjugate of Avidin-Antibody to oxidized LDL and removed by ECA with Biotin as the adsorbent. Pairs of affinity labels and their counterparts are known in the art and are disclosed, for example, in U.S. Patent 6,251,394, column 6, line 7 to column 8, line 53. Many specific ligands in the Avidin-specific ligand conjugate are known and many are available from commercial sources. Generally, such specific ligands are known by, or can be produced by persons with ordinary skill in the art, following established methods and without undue experimentation. For example, if the specific targeting ligand is a Mab, many of the molecular and cellular species, that are affinity targeted for removal have known Mabs that are specific to them and many of them are available commercially. For example, Mabs to CEA and other tumor antigens mentioned in the current application have been produced. Alternatively, such Mabs can be produced, without undue experimentation by those skilled in the art, by use of hybridoma Mab production techniques. As an alternative to administering affinity labels to the subject, the TSF, including TISF affinity labels as well as affinity labels specific to any endogenous or exogenous administered or invading cellular or molecular species, as detailed above (all such

molecular or cellular species will be referred to as MCS) can be incorporated in the ECA column, utilizing an ECA method that incorporates as adsorbents a FIRST and a SECOND adsorbent. For example the FIRST adsorbent may be Avidin or Protein A covalently bound to the matrix in the ECA column and the "SECOND" adsorbent may be a plurality of biotinylated antibodies (when Avidin is the FIRST adsorbent), or untreated antibodies (when Protein A is the FIRST adsorbent) that are specific to at least two TSF species, with, or without use of regeneration of the adsorbents. Use of double stage labeling of a tumor for radiolabeling a cancer was reported in H. P. Kalofonos et al., The Journal of Nuclear Medicine, Vol. 31, ~~no~~ No. 11, pp. 1791 -1796, Nov. 1990. The authors labeled a lung cancer with a conjugate of a Mab specific to the cancer antigen, that was conjugated to Strepavidin and administered Biotin-^{111}In to radio-label the tumor for diagnostic imaging. The authors did not disclose or hint to the possible use of such labeling for the ECA of molecular and cellular species. Matrixes other than Sepharose can be used to produce matrix-biotin conjugate for use in ECA. For example, G. Paganelli et. al. disclose the production of biotinylated Nitrocellulose and biotinylated Polystyrene (G. Paganelli et al., Int J. Cancer Suppl. 2, pp. 121-125, 1988.).

Please substitute the following paragraph for the paragraph beginning at page 44, line 18:

Following ECA on the Biotin adsorbent column, the subject is administered a VL or TL conjugated to Biotin (it should be realized that the use of Avidin and Biotin in the example can be reversed; with the sequence of Step l: Administration of Biotin-TAB, Step 2: ECA on Avidin column Step 3: Administration of TL-Avidin or VL-Avidin conjugate. Step 4 ECA on a Biotin column. It would also be realized that affinity pairs other than Avidin biotin can be

used instead of Avidin- Biotin pair (such as, for example: Hapten -Anti hapten antibody, Enzyme-substrate, Enzyme-Enzyme inhibitor.) The VL-Biotin conjugate can be Biotin-^{111}In and is prepared and administered to the subject according to Kalofonos et al., supra: Biotin covalently conjugated to diethylenetriaminepentaacetic acid (DPTA) is obtained from Sigma Chemical Co., St. Louis, Mo., and chelated to ^{111}In as described in ~~Kalafonos~~ Kalofonos et al. supra.

Please substitute the following paragraph for the paragraph beginning at page 45, line 6:

In Step 3, An anti-cancer drug, including, but not limited to one or more of the anticancer drugs disclosed in PCT WO 96/37516 as well as the anticancer drugs disclosed in U.S. Patent 5,527,528, incorporated herein by reference is incorporated in a liposome prepared as described in U.S. Patent 5,527,528, in particular, example 3, for the preparation of Avidin coated liposome omitting the last step of incubating the Avidin coated liposomes with biotinylated antibodies. Optionally the Avidin is bound to the Biotin on the liposome wall through a spacer. Suitable spacers are disclosed, for example, in ~~K~~ K. Hashimoto et al., Biochim Biophys Acta, Vol. 856 (3), pp. 556-65, April 25, 1986. The anticancer drug incorporated into the liposome in the Example is adriamycin.

Please substitute the following paragraph for the paragraph beginning at page 46, line 22:

In any of the ~~Examples~~ EXAMPLES 1-73, in the step of removal of specific species from the blood and for the application of the current invention, can be used for the adsorption- removal of any chemical species that may interfere with targeting, such as CA, CA-NAB, NAB, ATAA, or any molecular or cellular species that suppresses the Immune Destruction, or Non-Immune

destruction of the tumor (TSF, including TISF). The specific adsorbent is based on the method disclosed in U.S. Patent 5,753,227. Said patent is incorporated herein in its entirety. In accordance with the current example, intact antibodies, or antibody fragments, containing Fc, wherein said antibodies or Fc containing fragments are specific to an epitope on the molecular or cellular species that is to be removed from the biological fluid and BC, HC and TC are added to a Protein A extracorporeal column, such as Immunosorba® or Prosorba®, or any other Extracorporeal column that contains Protein A, bound to matrix or encapsulated, as disclosed for example in U.S. Patent 5,753,227, supra. Such antibodies or Fc containing fragments, in the current example are selected from antibodies, or fragments specific to the molecular and cellular species, that inhibit targeting or suppress tumor destruction as disclosed in the current application and in Hellstrom and Hellstrom and Botti et al., and ~~M R~~ M.R. Lentz ; Therapeutic Apheresis Vol. 3 (1) p 40-49, 1999.

Please substitute the following paragraph for the paragraph beginning at page 48, line 31:

In atherosclerosis (AS), IL-6 is elevated in the blood and systematically in the body, as well as AS plaques (ASP), particularly in unstable angina (UA). The adsorbent used in the ECA is a Mab specific to IL-6 (L.J. Cornfield and M.A. Sills, Eur. J. Pharmacol., Sept 4, 1991, Vol. 202(1) pp. 113-5. The Mab is incorporated in ECA device. Suitable devices and matrixes, including suitable methods for covalent and non-covalent binding of the Mab to the matrix are those described in U.S. Patent 4,813,924, example 13 and Fig 7, and U.S. Patent 6,039,946 when modified for use for only one Mab adsorbent. (It is noted that whenever Mab is mentioned, it includes fragments, including synthetic fragments and analogs.) The Mab can be encapsulated in a microcapsule or macrocapsule as described in U.S. Patent 6,039,946; see

in particular patent 6,039,946 col. 8 line 31 to col. 11, l. 60 and Fig 4. When Mab to IL-6 is encapsulated according to the original method of L. ~~Markus~~ Marcus et al., Am. Heart J., Vol. 110(1), part 1, July 1985, pp. 30-39, either the modified macrosphere encapsulation as used in the patent, or the method as described in Markus et al. can be used for the encapsulation of the IL-6 Mab. Preferably the Mab is covalently bound to cyanogen bromide activated cross linked agarose as described in the patent except that Mab to IL-6 is substituted for the Mab specific to LDL.

Please substitute the following paragraph for the paragraph beginning at page 51, line 28:

CIFs can also be removed by affinity adsorption with Mabs, utilizing the method described in B. L. Levine et al., J Hematotherapy, 1998, Oct, Vol. 7 (5), pp. 437, utilizing Mabs specific to one or more CIFs to specifically remove them. The system described in the paper above utilizes magnetic separation with antibody coated magnetic beads and a MaxSep® magnetic separator. Preferably, in the current Example, CIFs are removed using the Isolex 300i® system (Nexell Therapeutics), with the use of Mabs specific to the CIFs that are targeted for removal. The Isolex 300i® uses paramagnetic beads to which the specific Mab is bound, to remove the specific cells (CIFs), When it is desirable not to discard the CIFs, such as when it is desirable to collect them, the Isolex 300i® system provides a unique peptide release system to separate the cells from the Mab bound magnetic beads. Depending on the CIF to be removed either the removal system of the Isolex 300i® or another suitable peptide can be used, or the cells can be separated by other means, such as change in pH, mechanical shaking, change in ionic strength, or use of soluble cell receptor (epitope) including synthetic fragments of the receptor, including analogs. When in the Example the CIF targeted for removal is CD4+CD28null T cells, it is desirable not to reduce unnecessarily

general immune function of the organism being treated. In order to accomplish this aim, first CD4+ cells (both CD4+CD28null and CD4+CD28+) are removed by the Isolex 300 I® system, utilizing Mab specific to the CD4+ epitope. CD4+ cells are then eluted, as described above, The removed cells are then treated with beads that are bound to Mabs specific to the CD28 epitope, the bound cells are eluted: these are CD4+CD28+ T cells. These cells are returned to the subject being treated. The CD4+CD28null cells do not bind to the Mab-bound paramagnetic beads and are discarded, or collected for testing, if desired. Mabs specific to epitopes on CIFs can be produced by known hybridoma and other Mab production techniques, routinely used in the art. Many of the CIFs epitopes have known Mabs. For example, Mabs are known for CD28 epitope (S. Fretier et al., J Leukoc. Biol., 2002, Feb, vol. 71(2), pp. ~~298-94,~~ 289-94; S.D. Singh and C.G. Booth, J Immunol. Methods 2002, Vol. 260 (1-2) pp. 149-56). Anti CD4 Mab is also known. B. Sawltki et al., Euro. J. Immunol, March 2002, Vol. 32(3) pp. 800-9. CD4+ T cells can also be removed by an extracorporeal adsorption system described by H. Onodera et al., Ther. Apher., 1998, Feb 2(1), pp. 37-42.

Please substitute the following paragraph for the paragraph beginning at page 53, line 25:

~~In~~ This example is similar to any of the ~~examples~~ EXAMPLES 1-8, except that the tumor being treated with targeted TL, or visualized with targeted TL, is an ~~Ovarian Tumor~~ ovarian tumor, including but not limited to an ~~Ovarian Tumor~~ ovarian tumor with metastatic spread. The tumor ~~antigens~~ antigen is CA-125 and the targeting antibody is Mab OC 125 (Goff, BA et ~~al:~~ al, Br. J. Cancer, Vol. 70, pp 474-480, 1994).